



"ALEXANDER FLEMING"  
Biomedical Sciences Research Center

### **Technology Profiles for Commercial Exploitation**

1. Cellular Drug Screening Assay for Targeting the Cytoskeleton in Rheumatoid Arthritis
2. Autotaxin: a New Therapeutic Target in Chronic Inflammatory Disorders & Cancer
3. Targeting Post-transcriptional Regulators in Inflammation and Cancer
4. Towards Early Interventions for Cognitive Disorders
5. HO-1/IRF-3 – a new target for Infectious and Immune Diseases

Technology 1	Cellular Drug Screening Assay for Targeting the Cytoskeleton in Rheumatoid Arthritis
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### Brief Description

Fleming researchers have developed an actin-based cellular assay for drug discovery in treating Rheumatoid Arthritis (RA). The assay is using primary synovial fibroblasts (SF) isolated from the joints of proprietary arthritic transgenic mice overexpressing human TNF. The assay accurately quantifies the intracellular level of F-actin using fluorescently-labelled phalloidin. Reduced F-actin levels following treatment with a candidate compound indicates possible therapeutic potential for RA. The assay is fast, easy to perform with very good signal-to-noise ratio and excellent reproducibility and has the potential to develop into a high-throughput assay for screening candidate drugs in treating RA. The novelty of the assay lies in the fact that it is performed using primary arthritic cells; this offers the benefit of screening for drugs in a complex system which preserves the biological complexity of the arthritic microenvironment. The assay has already undergone preliminary validation with commercially available RA drugs, such as methotrexate.

### Background

The assay is based on our observations that the cytoskeleton is severely affected in synovial fibroblasts isolated from the joints of arthritic mice. SFs from these mice exhibit significantly more pronounced stress fibres and display enhanced motility and adhesion, properties which contribute to the invasion and destruction of the joint during the course of the disease. Notably, these findings were also corroborated by microarray expressions analysis that revealed a cluster of genes associated with the actin cytoskeleton to be deregulated in arthritic SFs from mice. These observations create the prospect that inhibiting actin polymerization or the accumulation of stress fibers and lowering cellular F-actin levels may comprise a new therapeutic approach for treating arthritis.

### Product Opportunity

- Use in drug screening
- To develop assay into an off-the-shelf screening tool for therapeutic agents for RA
- To develop a diagnostic assay for arthritis based on expression of genes that we have identified as being deregulated in RA

### Selected Patent Applications (Priority GR 20050100526, October 19 2005)

US 12/090,999  
Europe 06806493.0  
Japan 2008-535987

Type of partners/collaboration sought	Seeking partnerships with companies active in the following disease hubs: Arthritis (especially Rheumatoid, Juvenile and Osteoarthritis), Ankylosing Spondylitis, Lupus and Autoimmune Disease. Also seeking companies that are developing inhibitors of actin, actin modulators and/or actin-related proteins that may also have applications in cancer treatment.  Seeking R&D collaborations and licensing deals.
Research group:	Institute of Immunology, BSRC AI Fleming, Athens, Greece
Group Leader:	Dr George Kollias
E-mail:	techtransfer@fleming.gr
Web:	www.fleming.gr/en/investigators/Kollias/index.html
Group size:	17

Technology 2	Autotaxin: a New Therapeutic Target in Chronic Inflammatory Disorders & Cancer
<p>Autotaxin (ATX; ENPP2), originally isolated from the supernatant of melanoma cells as an autocrine motility stimulation factor, has been attracting increased attention as a possible pharmaceutical target since:</p> <ul style="list-style-type: none"> <li>• it is a secreted glycoprotein widely present in biological fluids</li> <li>• it controls production of Lysophosphatidic Acid (LPA), a phospholipid mediator acting via specific G protein coupled receptors to promote cell proliferation, migration and survival.</li> </ul> <p>Moreover, elevated ATX expression has been detected (from Fleming researchers and elsewhere):</p> <ul style="list-style-type: none"> <li>• in various forms of cancer (lung, colon, breast, ovarian, neuroblastoma)</li> <li>• in synovial fibroblasts from rheumatoid arthritis patients</li> <li>• in the frontal cortex of Alzheimer's patients</li> <li>• in the cerebrospinal fluid of multiple sclerosis patients.</li> </ul> <p><b>Tools &amp; Methods</b></p> <p>To explore the possible role of ATX and LPA in pathophysiology, our researchers have developed:</p> <ul style="list-style-type: none"> <li>• a conditional knock out mouse for ATX (healthy and fertile)</li> <li>• the corresponding obligatory full knock out mouse (exhibiting embryonic lethality due to aberrant angiogenesis and/or failure of neural tube closure)</li> <li>• a transgenic mouse overexpressing human ATX driven by the CMV promoter</li> <li>• 7 hybridoma cell lines expressing monoclonal antibodies against the human ATX</li> <li>• ELISA assays for ATX</li> <li>• Activity assays for ATX</li> </ul> <p><b>Specialist Expertise</b></p> <p>Our laboratories are specializing in a large variety of animal models of disease exploring a potential role of ATX in their pathogenesis through cell/tissue specific ATX ablation (with a multitude of Cre expressing transgenic strains driven by specific promoters):</p> <ul style="list-style-type: none"> <li>• Rheumatoid arthritis (spontaneous or induced)</li> <li>• Sepsis</li> <li>• Colitis - Colon cancer</li> </ul> <p>A special focus is devoted to lung pathogenesis and the corresponding animal models of</p> <ul style="list-style-type: none"> <li>• Pulmonary inflammation and fibrosis</li> <li>• Acute and ventilator induced lung injury</li> <li>• Asthma</li> <li>• Lung cancer</li> </ul>	
Type of partners/collaboration sought	Seeking R&D partnerships with companies who develop ATX inhibitors; wish to test ATX inhibitors in animal models of disease; seek new targets for chronic inflammatory disorders, neurodegeneration and cancer; wish to develop or license biological inhibitors of ATX, wish to (co)develop (or license) diagnostic/prognostic tools for chronic inflammatory disorders, neurodegeneration and cancer
Research group:	Institute of Immunology, BSRC AI Fleming, Athens, Greece
Group Leader:	Dr Vassilis Aidinis
E-mail:	techtransfer@fleming.gr
Web:	www.fleming.gr/en/investigators/Aidinis/index.html
Group size:	11

### Technology 3

### Targeting Post-transcriptional Regulators in Inflammation and Cancer

Fleming research has implicated specific RNA-binding proteins, such as Elavl1/HuR and AUF1, and signaling cascades modulating mRNAs containing AU-rich elements in pathological conditions such as inflammation and cancer. This work identifies post-transcriptional regulation of AU-rich mRNAs as a new target in treating these diseases.

#### Specialist Expertise

A number of animal models together with specific in vivo and vitro tests have been developed by Fleming researchers that can be used to identify and evaluate drug candidates targeting such post-transcriptional pathways. Specifically, a screening pipeline has been developed comprising the following steps:

- Transgenic (i.e. gene knockout) or pharmacological targeting of RNA-binding proteins in the mouse (obligatory or tissue restricted)
- Phenotypic, histological and cellular analyses in tissues of interest (innate and adaptive immune compartments, myeloid and lymphoid organs, solid tissues e.g. the intestine and the lung).
- Use of proprietary animal models of human disease (acute inflammation, inflammatory bowel diseases, systemic and organ specific autoimmunity, intestinal and lung cancer) for the phenotypic, flow-cytometric, serological and histological assessment of pathology.
- Ex vivo Cellular assays for the analysis of cellular processes and molecular mediators.
- Gene expression analyses including low, moderate and high throughput steady-state mRNA measurements, assessment of transcription, mRNA stability and translation, protein output.

The combination of approaches and animal models for post-transcriptional regulators and human disease results in a competitive advantage by ensuring the assessment of efficacy at numerous levels thus maximizing benefit.

#### Specialist Technologies & Materials

1. Transgenic mouse models for post-transcriptional modulation (Elavl1/HuR obligatory and tissue specific knock-outs; Elavl1/HuR conditional overexpressing mice; hnRNP/AUF1 obligatory and tissue specific knock-ins)
2. Animal models of (a) acute and chronic inflammation (endotoxemia, hepatitis, inflammatory bowel disease); (b) autoimmunity (arthritis, encephalomyelitis); (c) colon and lung cancer.

#### Patent Application

US 11/855,831(Priority date 15 September 2006)

Type of partners/collaboration sought	Seeking R&D collaborations with pharmaceutical and Biotechnology companies active in the areas of posttranscriptional regulation and/or in the development of anti-inflammatory or cancer therapeutics.
Research group:	Institute of Immunology, BSRC AI Fleming, Athens, Greece
Group Leader:	Dr Dimitris Kontoyiannis
E-mail:	techtransfer@fleming.gr
Web:	<a href="http://www.fleming.gr/en/investigators/Kontoyiannis/index.html">http://www.fleming.gr/en/investigators/Kontoyiannis/index.html</a>
Group size:	7

Technology 4	Towards Early Interventions for Cognitive Disorders
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Exploring the process of neuronal dysfunction, especially as it pertains to learning and memory and mobility deficits, which invariably occurs before neurodegeneration, Fleming research opens up opportunities to develop early interventions to a wide range of cognitive disorders.

The fruit fly *Drosophila melanogaster* offers an exceptional experimental system to address these molecular mechanisms at their elemental level and results are directly applicable to diseases because of the high degree of genetic and functional conservation with humans. We capitalize on *Drosophila*'s low cost, rapid breeding cycle and powerful molecular genetic arsenal. We have also developed methods to deliver small molecule drug candidates to the *Drosophila* brain, hence providing the fly as a cheap alternative drug screening tool. Our research also involves discovery of genes in *Drosophila* essential for learning and memory and their functional validation in the mouse, as a first step towards human applications.

### Specialist Expertise

Our specific expertise lies in the areas of Cognitive Disorders and Dementia and more specifically Tau related diseases such as Alzheimers and Frontotemporal Dementias, Learning Disorders like the Fragile X syndrome, Neurofibromatosis 1 and Attention Deficit Hyperactivity (ADHD) and age-related cognitive decline.

By investigating molecular mechanisms and signaling systems implicated in cognitive deficits in *Drosophila*, our multidisciplinary team focuses on understanding the molecular process(es) that allow information to be acquired by neurons and yield learning and memory formation, storage and recall. Specific expertise includes molecular biology, genetics, behavioral analyses, histology and molecular imaging, as well as effective and efficient methodology for drug delivery and evaluation in flies.

With respect to aggregation and pathogenicity of the Tau protein, our researchers have discovered several (currently unpublished) proteins that appear to modulate activity of kinases. Furthermore, novel compounds that inhibit metabotropic glutamate receptors and modulate cAMP levels are investigated as potential pharmaceuticals for the treatment of Fragile X syndrome.

### Specialist Tools

We are currently developing a high throughput assay to determine the pharmaceutical reversal of behavioural symptoms of mobility disorders such as Fragile X.

Type of partners/collaboration sought	Seeking collaborations with drug discovery companies with the following classes of candidate molecules: (1) Tau Disorders: Novel specific GSK3B inhibitors and inhibitors of Tau protein aggregates (2) Learning (Fragile X) Disorders: membrane permeable cAMP analogues, novel Glutamate receptor blockers and GABA receptor agonists. Also seeking partnerships with companies wishing to test their drugs in our newly developed assay.
Research group:	Institute of Molecular Biology & Genetics, BSRC AI Fleming, Athens, Greece
Group Leader:	Dr Efthimios Skoulakis
E-mail:	techtransfer@fleming.gr
Web:	<a href="http://www.fleming.gr/en/investigators/Skoulakis/index.html">www.fleming.gr/en/investigators/Skoulakis/index.html</a>
Group size:	14

**Brief Description**

Fleming's researchers have revealed a novel, major role for Heme Oxygenase-1 (HO-1) in the regulation of interferon- $\beta$  (IFN- $\beta$ ) production. This action is attributed to HO-1's direct interaction and activation of IRF-3, a key transcription factor in the signalling pathways that activate IFN- $\beta$  production. These results point to an early role for HO-1 in the activation of IFN type I pathways. Considering IFN- $\beta$ 's key role in innate and adaptive immune responses, this work identifies the HO-1/IRF-3 interaction as a new target in developing effective therapeutic interventions in a range of infectious and immune diseases. A screening assay to test for compounds which specifically modulate the interaction between HO-1 and IRF3 has also been developed by Fleming researchers. Partnerships are thus sought in developing compounds that modulate the HO-1/IRF-3 interaction, in myeloid or fibroblast cells for instance, that may be of therapeutic potential.

**Background**

HO-1 is a key cytoprotective, anti-oxidant and anti-inflammatory molecule. Thus far, HO-1's physiological functions have been associated with its enzymatic activities in heme catabolism. IRF-3 is a key transcription factor in the production of IFN- $\beta$  in response to specific stimuli such as infection. The transcriptional activation function of IRF-3 is linked to its regulated phosphorylation.

Using a conditional gene knockout approach in mice, Fleming researchers have ablated HO-1 expression in myeloid cells to show novel HO-1 functions, as follows:

- (I) HO-1 deficiency resulted in impaired IFN- $\beta$  production in response to TLR3/4A.
- (II) An interaction of HO-1 with IRF-3 was described which resulted in IRF-3 activation via phosphorylation.
- (III) A requirement of HO-1 in the generation of IFN- $\beta$  dependent death signals in response to TLR3 activation and microbial or viral infection was shown.
- (IV) HO-1 deficiency resulted in an exacerbated experimental autoimmune encephalomyelitis (multiple sclerosis) phenotype in mice that is dependent on endogenous IFN- $\beta$  production.

This novel HO-1 involvement seems functionally distinct from the late anti-inflammatory response activated by HO-1 (CO pathway).

**Patent Protection**

US Provisional 61/153,419, 18<sup>th</sup> February 2009

Type of partners/collaboration sought	Seeking collaborations and license deals with drug discovery and pharmaceutical companies working in the infectious disease and immune disease fields. Specifically we are interested in companies that have or are developing HO-1 inhibitors and activators, IRF-3 modulators and TLR-3 and TLR-4 based therapeutics.
Research group:	Institute of Immunology, BSRC AI Fleming, Athens, Greece
Group Leader:	Dr George Kollias
E-mail:	techtransfer@fleming.gr
Web:	<a href="http://www.fleming.gr/en/investigators/Kollias/index.html">www.fleming.gr/en/investigators/Kollias/index.html</a>
Group size:	17